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Meta-analysis reveals the combined effects of microplastics and heavy metal on plants

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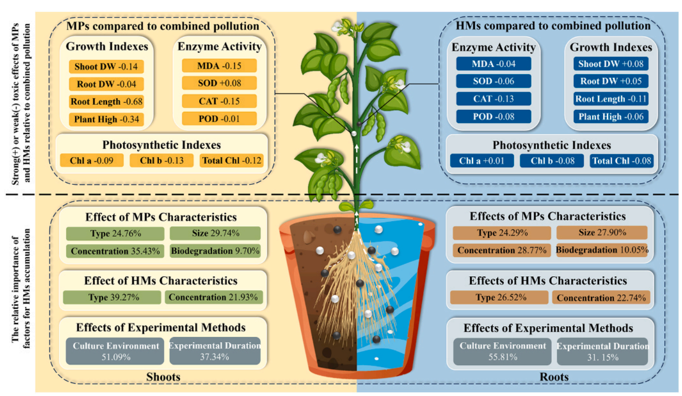
HIGHLIGHTS

- Impacts of combined pollution of MPs And HMs on phytotoxicity were evaluated.
- MPs can exacerbate plant oxidative stress damage induced by HMs.
- The concentration and size of MPs significantly affect HMs accumulation in plants.
- MPs biodegradation has a strong interaction with various experimental conditions.

GRAPHICAL ABSTRACT



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ABSTRACT

The combined pollution of microplastics and heavy metals is becoming increasingly serious, and its effects on toxicology and heavy metal accumulation of plants are closely related to crop yield and population health. Here, we collected 57 studies to investigate the effect of microplastics on heavy metal accumulation in plants and their combined toxicity. An assessment was conducted to discover the primary pollutant responsible for the toxicity of combined pollution on plants. The study examined the influence of microplastic characteristics, heavy metal characteristics, and experimental methods on this pollutant. The results showed that combined toxicity of plants was more similar to heavy metals, whereas microplastics interacted with heavy metals mainly by inducing oxidative stress damage. Culture environment, heavy metal type, experimental duration, microplastic concentration and microplastic size were the main factors affecting heavy metal accumulation in plants. There was a negative correlation between experimental duration, microplastic concentration and microplastic size with heavy metal accumulation in plants. The interactions among influencing factors were found, and microplastic biodegradation was the core factor of the strong interaction. These results provided comprehensive insights and guiding strategies for environmental and public health risks caused by the combined pollution of microplastics and heavy metals.

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1. Introduction

Plastic products have been widely used in daily life and agricultural production [1,2]. Through weathering mechanisms, photodegradation and biodegradation, plastic wastes can be degraded into microplastics (MPs) with a diameter of less than 5 mm, which exist in the natural environment for hundreds of years [3,4]. Currently, MPs have been detected in large quantities in terrestrial and aquatic ecosystems, even in the less visited Antarctic, possibly due to inappropriate treatment and lack of control of plastic waste [5]. The situation is likely to continue to worsen given the constant production of plastic waste, which in the natural environment expected to reach 12,000 million tons by 2050 [6]. As a result, there has been widespread concern about the ecological and environmental concerns posed by MPs.

Due to large surface area and strong hydrophobicity, MPs are often thought to be effective carriers of other pollutants [7]. Meanwhile, the earth's crust background value and the discharge of heavy industry waste provide the necessary conditions for the combined pollution of MPs and heavy metals (HMs) [8]. The quality and safety of plants are closely related to human health, so the effects of MPs on HMs have received extensive attention in the field of phytotoxicity. At present, studies on the effects of combined pollution of MPs and HMs on plant biology, oxidative stress and photosynthetic have been reported one after another, which is relatively comprehensive [9,10]. These findings usually indicated that the combined toxicity effects of MPs and HMs have intensified than either alone. Some studies have shown that MPs can absorb more pollutants and be ingested by organisms, resulting in more serious negative biological effects through "Trojan horse effect" [11]. However, the MPs that can enter the interior of the plant are far fewer than those attached to the root surface, and have strong size limitations [12]. In addition, although some studies have indicated that MPs can induce oxidative stress damage in plants, but whether they also play an important role in combined pollution with HMs has also been less studied. Meanwhile, it is still unclear whether they created this effect independently or interactively. Therefore, more methodical investigation is required in order to comprehend the mechanism in the combined effects of HMs and MPs on plants.

However, the current research results on the effects of MPs on HMs accumulation in plants are uneven. Dong et al. [13] found that MPs could inhibit the uptake of arsenic (As) by rice via inhibiting root activity and reducing the iron plaques of root coating. In contrast, Jiang et al. [11] suggested that the presence of nanoplastics increased the expression of aquaporin-related genes in rice and impaired its detoxification pathway, thereby inducing an increase in As accumulation. This suggested that differences in MPs characteristics, HMs concentrations, or designed experimental conditions might have different effects on phytotoxicity. Furthermore, this also made it impossible to obtain a comprehensive understanding by comparing individual cases, which brings challenges for comprehensive assessment of phytotoxicity caused by HMs combined with MPs. In response to the above problems, some academics have started conducting comparative studies on various experimental conditions to explore the possible factors affecting the effects of MPs and HMs combined pollution on phytotoxicity. They compared the toxicological effects of different type [14], size [15], concentration [16] and biodegradation [17] of MPs, type [18] and concentration [19] of HMs on plants. However, the limitations of the laboratory study led them to focus only on one of these parameters for comparison. As a result, the factors covered in the comparative study are not comprehensive enough to reach a universal conclusion, and it is also impossible to judge the complex interaction between various factors. Nevertheless, the pollution status of MPs and HMs in the natural environment is often more complicated than the experimental design, resulting in different MPs characteristics, HMs conditions, pollution environment and pollution duration often may exist simultaneously. With the extension of time, MPs can be aged to provide more sites to adsorb HMs [20]. Biodegradable MPs also have a faster aging rate than conventional MPs [21]. This suggested that there might be interaction between various factors to affect their phytotoxicity, which should be investigated urgently. If the relevant data related to the above of previous researches can be incorporated into a unified model for systematically evaluating the phytotoxicity caused by HMs and MPs, the problems caused by the differences in experimental conditions and methods may be eliminated. It should be noted, although there have been two meta-analysis reports similar to this topic in recent years, they mostly focus on HMs bioavailability [22] or only consider a particular HMs, specifically Cd [23]. Therefore, related research on the topic is still very poor, and there is still a great need to investigate the combined effects of MPs and HMs on plants in order to discover the interaction among various influencing factors and exploring the potential mechanism of combined pollution in a more comprehensive manner.

Here, a meta-analysis was conducted based on a global database to comprehensively and systematically quantify the combined effects of MPs and HMs on plants. At the same time, random forest and Geodetector models was used to further screen the key factors affecting HMs accumulation in plants by MPs and explore their interactions, which are difficult to achieve in laboratory studies. The aims of this research were to: (1) Comprehensively exploring the possible mechanism of phytotoxicity caused by combined pollution of MPs and HMs through data analysis; (2) Analyzing the effects of different MPs characteristics and experimental conditions on HMs accumulation through data integration, and further determining the main influencing factors; (3) Exploring the interaction between different influencing factors through mathematical models. This will help to understand the complex mechanisms underlying the toxicological effects of MPs and HMs on plants, providing a certain reference value for alleviating the environmental impact of MPs.

2. Literature and methods

2.1. Literature search and screen

Prior to January 9, 2024, all articles published in the Web of Science Core Collection, ScienceDirect, and Springer databases were searched using the keywords "microplastic", "nanoplastic", "plastic particle", "heavy metal" and "plant". Subsequently, two researchers independently screened the literature by reading the title and abstract respectively to guarantee that the articles included were accurate and universally applicable. For controversial articles, the two researchers would confer together to determine whether to retain them. The 102 articles were eventually used for subsequent screening. To further increase the relevance and comparability of the data, the articles were screened according to the following criteria: (1) Delete duplicate references from three databases. (2) Remove lower plants such as algae. (3) Remove pollutants that contain not only MPs and HMs. (4) Must include one of the indexes of plant growth (dry weight, root length and plant high), enzyme activity (MDA, SOD, CAT, POD, H_2O_2), photosynthetic (chlorophyll a, chlorophyll b and total chlorophyll) and HMs accumulation. (5) The experimental group (added HMs and MPs) and the control group (without HMs and MPs) need to coexist, and the samples need to have more than three replicates. Finally, a total of 57 laboratory research articles using virgin MPs were included, and 605 groups of data sets were obtained. The specific number of articles contained in each metric can be viewed in Fig. S1.

2.2. Pre-treatment of data

The mean and standard deviation (SD) of each index in publications were extracted. If there was only standard error (SE), Eq. 1 was used for conversion, where n is the number of samples. If only the mean was included, the SD should be calculated as 10 % of the mean. GetData Graph Digitizer (v.2.26) was used to extract the data if it existed in a graph rather than a table. For the purpose of subgroup analysis, indicators are grouped according to the following criteria: (1) MPs size was divided into ≤ 1, 1–100 and ≥ 100 μm. (2) MPs concentration was divided into≤ 100, 100–1000 and ≥ 1000 mg/kg or mg/L. (3) MPs type was subsequently also divided into conventional and biodegradable. (4) HMs concentration was divided into≤ 10, 10–50 and ≥ 50 mg/kg or mg/L. (5) Experimental duration was divided into ≤ 30, 30–60 and ≥ 60 d.

$$SD = SE \times \sqrt{n} \quad (1)$$

2.3. Meta-analysis

Meta-analysis of random effects model was implemented by MetaWin2. Response ratio (RR) was used as an effect size pairing to compare the experimental and control data (Eq. 2). The RR was then normalized by a natural logarithm transformation, which could facilitate equal treatment of the deviations in the numerator and denominator to prevent bias caused by a small sample size (Eq. 3) [24].

$$RR = \frac{\overline{x}_e}{\overline{x}_c} \quad (2)$$

$$\ln RR = \ln\frac{\overline{x}_e}{\overline{x}_c} \quad (3)$$

where $\overline{x}_e$ and $\overline{x}_c$ stand for the means of the experimental and control groups, respectively.

Hedges et al. [25] showed that lnRR was approximately normal distribution, hence the variance can be calculated by Eq. 4. The heterogeneity of different categories of lnRR was calculated using the 95 % confidence interval (95 %CI) under the random effects model (Eq. 5). If 95 %CI overlapped with zero, the effect was considered insignificant, while no overlap indicated that the effect was statistically significant.

$$V = \frac{SD_e^2}{n_e\overline{x}_e^2} + \frac{SD_c^2}{n_c\overline{x}_c^2} \quad (4)$$

$$95\%CI = \ln RR + 1.96 \times \sqrt{V} \quad (5)$$

where SD_e and SD_c were the SD of the experimental and control groups, while n_e and n_c were the sample numbers for them.

The between-group Q test was used to compare the heterogeneity of each effect size among groups. Significant Q values (Q_B) indicated differences among the groups ($P < 0.05$). In addition, Rosenthal's method was used to test the publication bias of the included articles. To ensure the reliability of the results, no significant publication bias was required in most variables, which meant that Rosenthal's fail-safe number must be greater than 5 n + 10 (n was the number of observations) [26]. Detailed data of fail-safe numbers for each index in this study could be viewed in Table S1.

2.4. The main influencing factors and their interaction

Linear regression analysis between the influencing factors was performed using Origin 2024 software (OriginLab Corporation, Northampton, MA, USA). In order to compare the relative importance of each factor, a random forest model was chosen and its analysis was carried out in R 4.2.2 using the "randomForest" and "rfPermute" packages. GeoDetectors (http://www.geodetector.cn/) were then used to explore the interactions between the various influencing factors, a detailed classification of which was shown in Table S2.

3. Results and discussion

3.1. Comprehensive effects of combined pollution on plant

As shown in Fig. 1, combined pollution of MPs and HMs could inhibit biomass and length(high) of plant shoots and roots, as well as chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (Total Chl) content, which were not conducive to plant health. Roots serves as the first safety barrier for plants against pollutants, whether in water or soil culture environment [27]. The growth conditions of roots directly affect the absorption and migration of pollutants, which would indirectly affect shoot growth and photosynthesis through the toxicity of pollutants. The results of meta-analysis showed that both MPs and HMs could reduce the roots biomass (Fig. 1a and b), especially HMs, which also caused the decrease of root length, shoot biomass and plant high (Fig. 1b). The researchers revealed that HMs reach the plant roots with water as they absorb water through diffusion or by pulling with transpiration, and then bioavailable HMs ions were effectively absorbed through membrane transporters [28]. Some HMs, like As and Cr, can compete with nutrient elements for the same transmembrane proteins, affecting nutrients absorption [29,30]. Many HMs, such as Cu, Cd, Pb, As, affect the structure and function of many proteins by reacting with sulfhydryl groups or displacing coordination ion on proteins, and can also cause oxidative stress damage in plants by inducing ROS production [31,32]. All these reactions affect the growth and photosynthesis of plants, resulting in the reduction of roots and shoots biomass and photosynthetic pigments. In contrast, MPs can disrupt the adsorption on the root surface of plants, affecting the uptake of water and nutrients, and causing oxidative stress damage [33,34]. Limited root permeability can inhibit plant growth and photosynthetic rates, further limiting root growth [35,36]. In addition, although some studies had shown that MPs could enter plants through mechanical wear and migrate to the shoots with transpiration, they were mainly concentrated in the roots [12], which may explain why MPs pollution alone had an effect on roots biomass but not on the shoots. However, it was worth noting that although researchers have different perspectives on how MPs and HMs inhibited plant growth, the induction of oxidative stress damage appears to be recognized as the common mechanism by which they affect plant health.

Therefore, the enzyme activities of plant were also analyzed by meta-analysis, which revealed that MPs, HMs and their combined pollution did change them (Fig. 1). Moreover, the effect of MPs on enzyme activity was more similar to that of combined pollution, which increased the activities of MDA, SOD, CAT, POD and H_2O_2 (Fig. 1a and c). In contrast, HMs did not affect SOD and POD as combined pollution did (Fig. 1b). MDA, CAT, H_2O_2, SOD and POD are all oxidative stress indexes in plants, which can be changed under environmental stress. MPs and HMs as pollutants can create environmental stress, their combined pollution would lead to increased activity of antioxidant enzymes such as SOD, CAT and POD [37], which was consistent with the obtained results (Fig. 1c). Both CAT and POD are enzymes that decompose H_2O_2, the difference is that CAT catalyzes the decomposition of high concentration H_2O_2 in tissues, while POD mainly works on the low concentration due to inhibition by high concentration H_2O_2 [38]. Therefore, the higher activity of CAT indicated that plants might have strong oxidative stress damage under the combined pollution of MPs and HMs. MDA is the decomposition product of polyunsaturated fatty acids in biofilms, which is one of the main indicators to measure the degree of oxidative stress in plants [39]. The increase of MDA activity supported the hypothesis that plants were under high levels of oxidative stress, which would explain the lower activity of POD than CAT. Interestingly, SOD is the first line of defense for the plant's antioxidant defense system and can eliminate a large number of ROS [40], but its activity was lower than CAT under combined pollution of MPs and HMs. Some studies have shown that MPs may reduce SOD activity in plants by impeding the absorption of trace elements including Fe, Mn, and Cu necessary for SOD isoenzyme production [41]. In addition, Gao et al. [12] believed that this may be related to the electrification of MPs.

In conclusion, the effects of combined pollution on various indexes of plant were more similar to those of HMs, except for SOD and POD activities. Therefore, the relationship between HMs accumulation in plants and various indexes would be further analyzed. But compared with HMs,

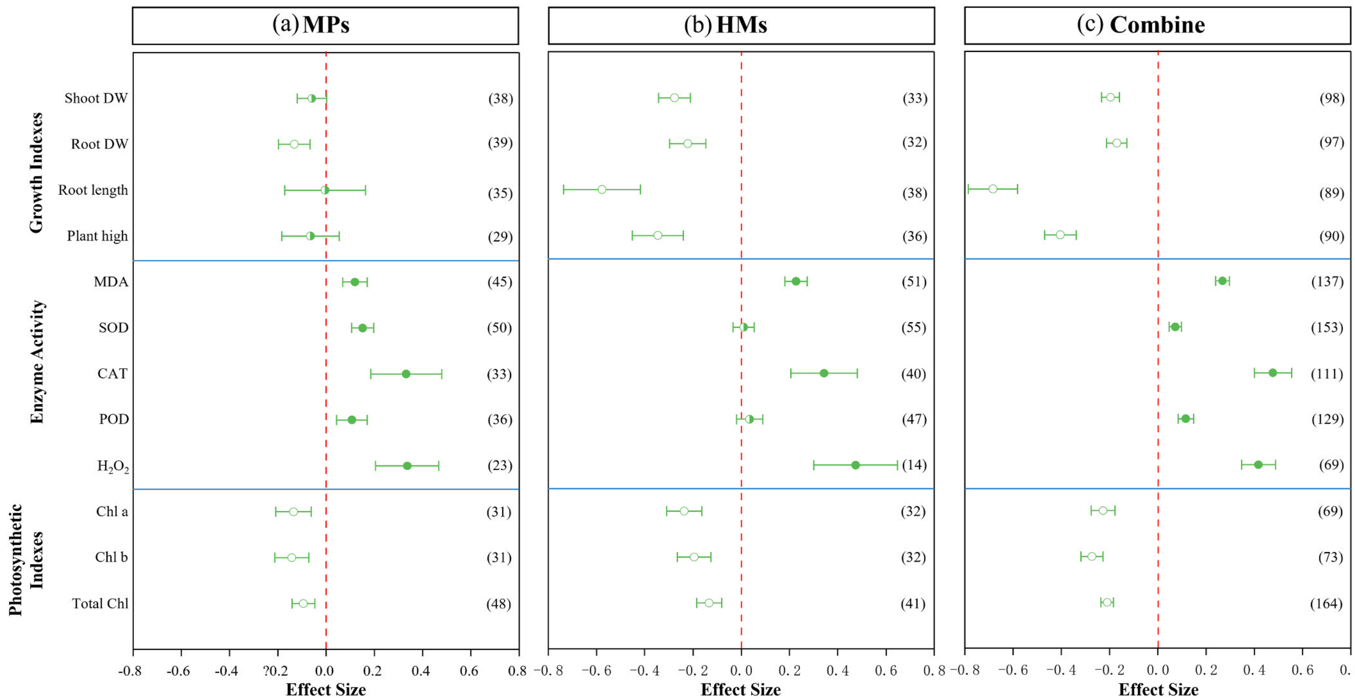

Fig. 1. Effects of MPs, HMs and their combined pollution on growth, enzyme activity and photosynthetic indexes of plant. The green points represent the effect size, and the error bars on either side indicate a 95 % confidence interval (CI) for the effect size. Red line, which effect size is zero, is used to help determine changes in the effect. The overlap of the 95 % CI with the red line means a neutral effect, a positive effect entirely to its right and a negative effect entirely to its left. Solid points represent a positive effect, while semi-open and open points represent a neutral and negative effect, respectively. The numbers in parentheses are the amount of data included in the analysis. DW, dry weight; Chl, chlorophyll.

combined pollution had stronger inhibitory effects on plant high, root length and chlorophyll content, and had higher MDA and CAT activities of plant (Fig. S2), which might be related to the synergistic enhancement effect caused by oxidative stress damage inducing from MPs.

3.2. Correlation between HMs accumulation and plant physiological indexes

The correlation results showed that there was a positive correlation between HMs accumulation and water content in roots (Fig. 2b), while HMs accumulation in shoots was highly positively correlated with that in roots (Fig. 2c) but not with water content (Fig. 2a). However, there was no statistically significant correlation between HMs translocation factor from root to shoot and MPs concentration, MPs size, HMs concentration and experimental duration (Fig. S3). These results revealed that MPs did not seem to affect HMs translocation from root to shoot, and their effect on HMs accumulation might be attributed to changes in HMs absorption by root, which was accompanied by water absorption. Meanwhile, the HMs accumulation in plant could decrease dry weight and length(high) of plant shoots and roots, and reduce the SOD and chlorophyll content (Fig. 2d). Furthermore, dry weight and length(high) of shoots and roots and chlorophyll content were negatively correlated with enzyme activity indexes such as MDA, CAT and H_2O_2 (Fig. 2d). These results suggested that HMs accumulation could inhibit plant growth and photosynthesis, which was closely related to the reduction of antioxidant ability and the formation of oxidative stress damage.

MPs are electronegative and often thought to be highly hydrophobic, similar to the cellulose cell wall of the root cell, allowing them to attach to the surface of the root. At the same time, MPs can also form a complex with root secretions to form a hydrophobic film on the root surface to inhibit water absorption by the root [42]. Even if MPs penetrate into the root cells through the adventitial root zone or epidermal tissue, they can accumulate in the cells and affect the links between cells, thus impeding the absorption of water and nutrients and the transport to other cells [43]. Based on the high correlation between HMs accumulation and water content, it is reasonable to speculate that MPs can inhibit HMs uptake through the aforementioned mechanism. At the same time, the resulting hindered uptake of water and nutrients often leads to water stress and nutrient limitation, inducing oxidative stress damage. The appearance of oxidative stress not only causes the death of plant tissue cells, but also leads to the abnormal distribution of auxin [32]. Low concentrations of auxin are necessary for normal root growth, but oxidative stress can induce polar auxin transport in plants, causing it to accumulate at the root tip and inhibiting root elongation [44]. Oxidative stress also interferes with the electron transport mechanisms that occur in chloroplast and mitochondrial membranes, inhibiting plant photosynthesis, reducing sugar metabolism and nutrient content, which therefore hinders root elongation [45]. In addition, HMs can also decrease the photosynthetic efficiency of plants by enhancing the activity of chlorophyll enzymes and inhibiting the biosynthesis of chlorophyll [46]. Combined with the correlation analysis results of plant physiological indexes (Fig. 2d) and the above discussion, it was evident that oxidative stress damage induced by combined pollution of MPs and HMs could inhibit plant growth and photosynthetic efficiency, and the reduction of photosynthetic efficiency would subsequently lead to abnormal plant growth indicators, creating a vicious circle. However, compared with MPs, the mechanisms of HMs inhibiting plant growth were more diverse, potentially explaining why the effects of combined pollution were more similar to HMs. Based on the high similarity between the effects of combined pollution and HMs on phytotoxicity, as well as many speculations about the effects of MPs on HMs absorption in plants, it was necessary to further conduct subgroup analysis on MPs characteristics, HMs characteristics and experimental methods to explore how these influencing factors affect HMs accumulation in plants.

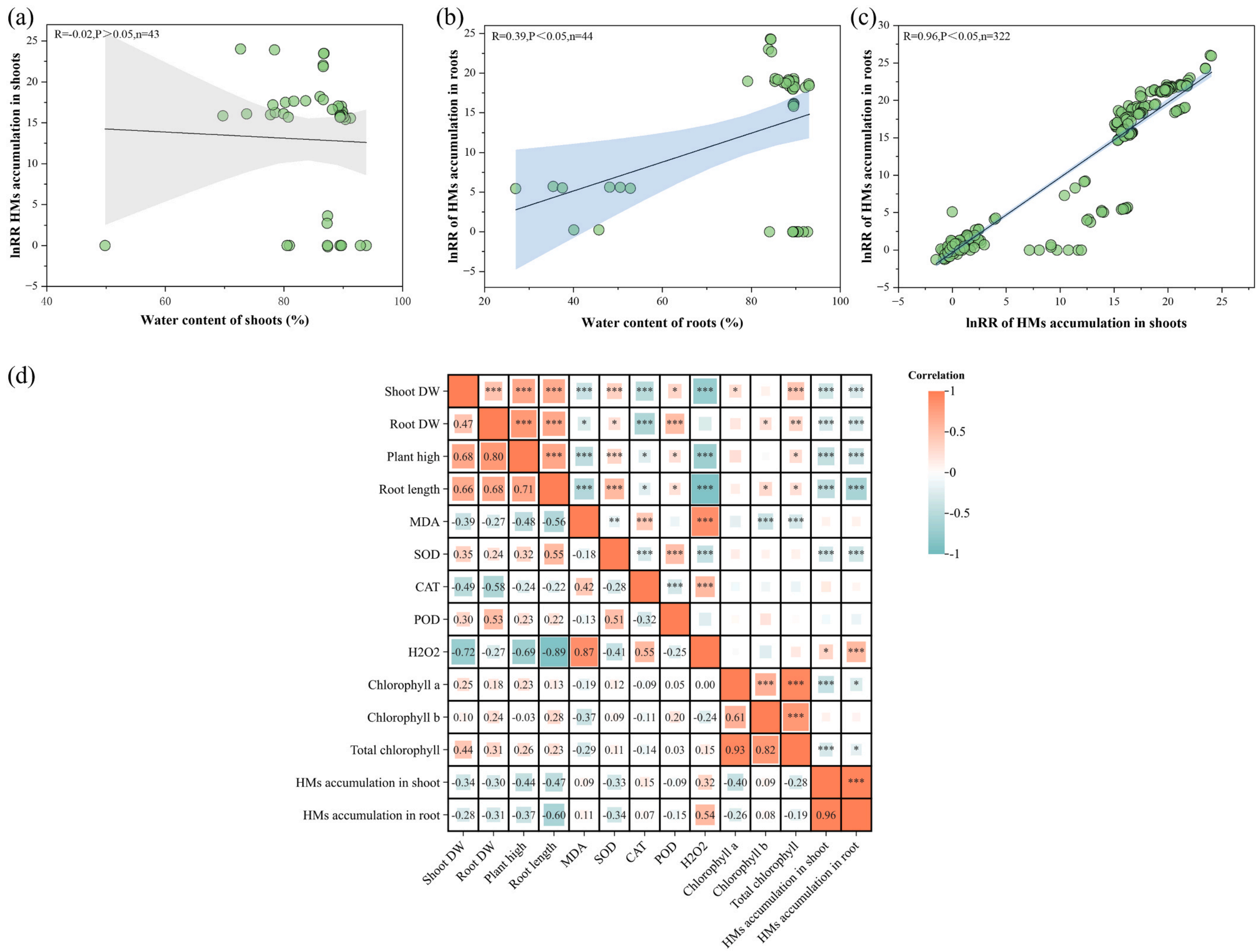

Fig. 2. The correlation analysis between HMs accumulation with water content in shoots (a), HMs accumulation with water content in roots (b), HMs accumulation in shoots and roots (c), and HMs accumulation and each physiological index of plants in roots and shoots. R is the correlation coefficient, whose positive and negative indicates the direction of the effect, and the size indicates the strength of the effect. $P < 0.05$ indicates statistical significance. n indicates the amount of data included in the analysis. * $P < 0.05$, * * $P < 0.01$, * ** $P < 0.001$.

3.3. Different responses under variable experimental conditions

3.3.1. MPs characteristics

Characteristics such as type, size, concentration and biodegradation of MPs were selected as factors affecting HMs accumulation in plants, regardless color, shape and aged, because the included researches were laboratory studies that made the selected MPs primitive, spherical and transparent. According to the results of subgroup analysis, PS, PE and PLA could decrease HMs accumulation in shoots and root, while PVC could decrease HMs accumulation in shoots (Figure 3a1 and b1). Chen et al. [9] speculated that one possible reason was that it can be attached to the root surface, hindering the physical contact between HMs and roots, and subsequently interfering with HMs uptake by roots based on its hydrophobicity. However, this inhibition mechanism seemed to be related to MPs size, as MPs with a diameter of less than 2 μm had been reported to penetrate vegetable roots and pass through plant tissue [47]. Therefore, MPs size was also used as one of the influencing factors for subgroup analysis. Furthermore, the lack of inhibitory effects in PMMA (1.15–1.19 g/cm^3) and PA (1.12–1.15 g/cm^3) might be attributed to their high density, which results in poor buoyancy and problematic migration with water. However, PVC (1.10–1.30 g/cm^3) and PLA (1.25–1.28 g/cm^3) also have very high densities, but they exhibit significant variation. PVC has -Cl groups, which make it more polar and simpler to interact with water [48]. PLA includes a high concentration of nitrogen, which can offer favorable nutritional circumstances for microbes and have a greater impact on microbial composition than other MPs [49].

The subgroup analysis of MPs size showed that although different sizes all inhibited HMs accumulation in plants, the inhibition effect was stronger at larger size (Figure 3a2 and b2). When MPs size decreases, their specific surface area correspondingly increases, providing them with more binding sites for HMs adsorption. Furthermore, MPs with small size can enter the plant through the cracks in roots, and then reach the shoots through the xylem with transpiration [50]. This also caused the HMs absorbed on their surface to accumulate in the shoots and roots, which relatively reduced their inhibition effects on HMs accumulation in plants [51]. In theory, the increase in MPs concentration should have a synergistic effect with MPs size, since more MPs would enter the plant. However, subgroup analysis indicated that MPs concentration actually had a negative effect on HMs accumulation in plants (Figure 3a3 and b3). This might be because that although MPs could enter the plant through the roots, too much MPs would block root cracks, so that it inhibited HMs absorption [52]. Meanwhile, the blocked roots also inhibited the absorption of water and nutrients [53]. This would aggravate the induced by MPs, inhibiting plant photosynthesis and resulting in root growth restriction [54,55]. The weakening of root

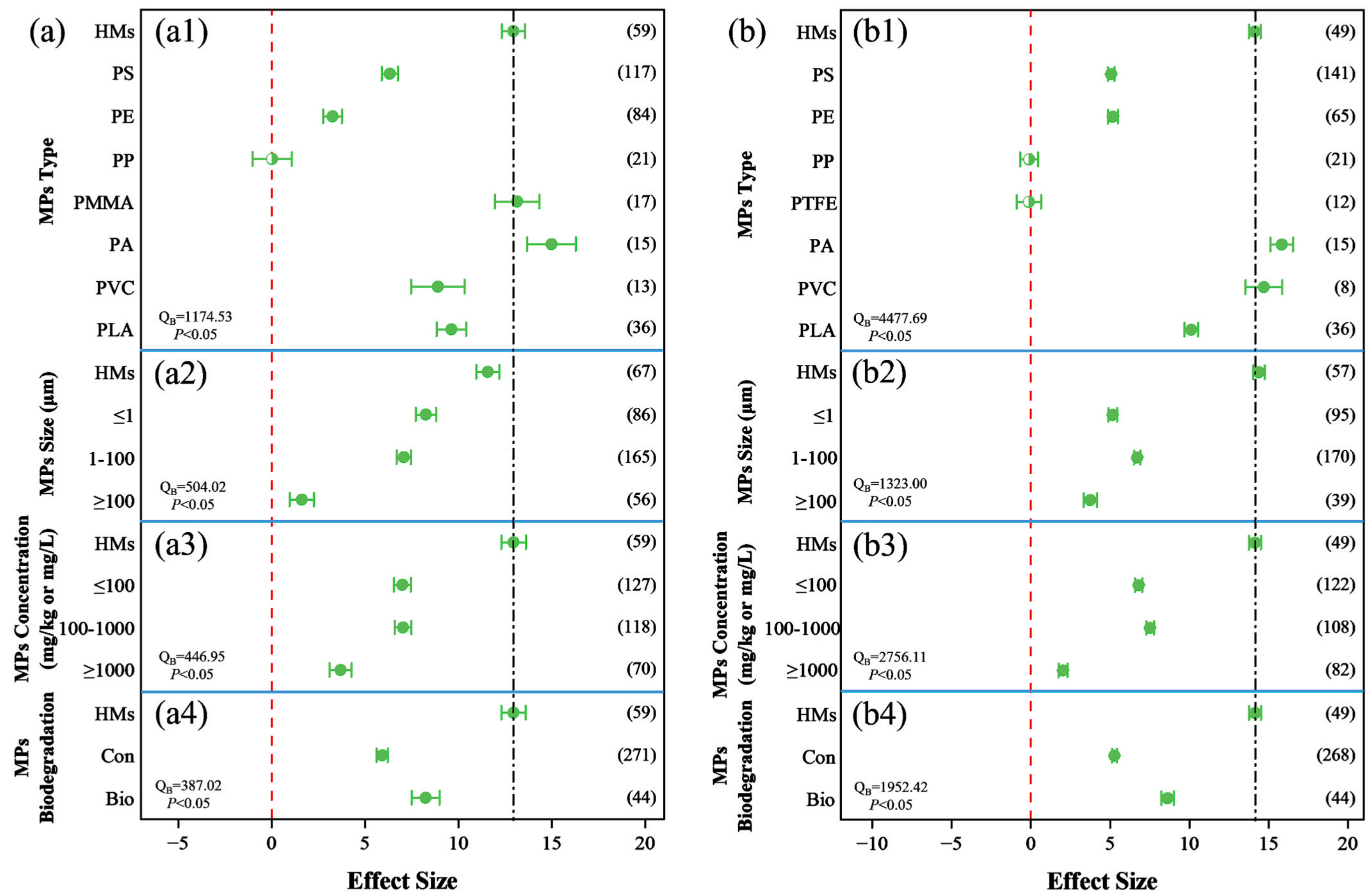

Fig. 3. Effect size of MPs characteristics on HMs accumulation in shoots and roots. The green points represent the effect size, and the error bars on either side indicate a 95 % confidence interval (CI) for the weighted mean difference combining the effect size. Red line, which effect size is zero, is used to help determine changes in the effect. Black line, which effect size for single HMs, is used to help compare its differences with single MPs and combined pollution. The overlap of the 95 % CI with the red line means a neutral effect, a positive effect entirely to its right and a negative effect entirely to its left. Solid points represent a positive effect, while semi-open and open points represent a neutral and negative effect, respectively. The numbers in parentheses are the amount of data included in the analysis. Q_B represents inter-group heterogeneity, and $P < 0.05$ indicates statistical significance. Con, conventional; Bio, biodegradable.

elongation reduced the area of roots that can adsorb HMs, which is not conducive to HMs accumulation in plants.

Meanwhile, the results suggested that conventional MPs might inhibit HMs accumulation in plants more effectively than biodegradable MPs (Figure 3a4 and b4), possibly due to the differences in their effects on the HMs bioavailability. It has been shown that biodegradable MPs were potential carbon sources in soil, which may change the soil microbial community and related soil properties, contributing to the improvement of HMs bioavailability [49]. In addition, our previous study had similarly shown that biodegradable MPs could contribute to improve As bioavailability in paddy soils by promoting an increase in the abundance of microorganisms with functional genes for As reduction and methylation [56]. However, HMs were absorbed by plants together with water and accumulate in plants (Fig. 2b). But MPs would adsorb on the root surface to hinder water absorption, and may compete with HMs for adsorption sites owing to electric charge [40]. As a result, although HMs bioavailability was increased, they cannot be absorbed by plants, which may explain the apparent relative reduction in HMs accumulation caused by combined pollution of MPs and HMs (Figure 4a1 and c1). In addition, biodegradable MPs are also more likely to be degraded into smaller nanoplastics than conventional MPs [49], which will also decrease their impact on HMs accumulation in plants.

At the same time, the effects of MPs characteristics on plant growth indexes were also the direction to be explored. Unfortunately, due to the limitation of data, only the effects of MPs types on various plant growth indexes fulfill the safety factor standards. Therefore, only the effects of MPs types on plant growth indexes were classified by subgroup analysis. The results indicated that different MPs had different effects on plant growth indexes. PE and PVC inhibited the growth of plant shoots and roots, while PS inhibited shoots growth and root biomass. Although PLA, PP and PMMA also had effects on shoots biomass, plant high and root length, respectively, the small number of trials may indicate that the results were not representative (Fig. S5). The inhibitory effects of MPs on plant growth were caused by a variety of mechanisms. In addition to impeding water and nutrient absorption, MPs may cause oxidative stress damage, restrict photosynthesis, and alter the structure and metabolic function of inter-root microbial communities, changing the environment for root growth and overall plant life [57]. Therefore, further study in these areas will pave the way for more subgroup analysis of MP characteristics.

3.3.2. HMs characteristics

Unlike the MPs concentration, HMs concentration did not appear to have a significant negative correlation with HMs accumulation in shoots and roots, but inhibition was more pronounced at $\geq$ 50 mg/kg or mg/L (Figure 4a2 and c2). Plants have corresponding self-protection mechanisms while absorbing HMs. Plant roots can prevent HMs accumulation in plants by secreting rhizosphere substances or forming iron plaques [11,27]. Even if HMs was absorbed by plants, it could also chelate HMs with high-affinity ligands such as phytochelatins, organic acids and amino acids in the cytoplasm [58]. This process helps reduce the oxidative stress damage in plants, allowing them to tolerate the damage

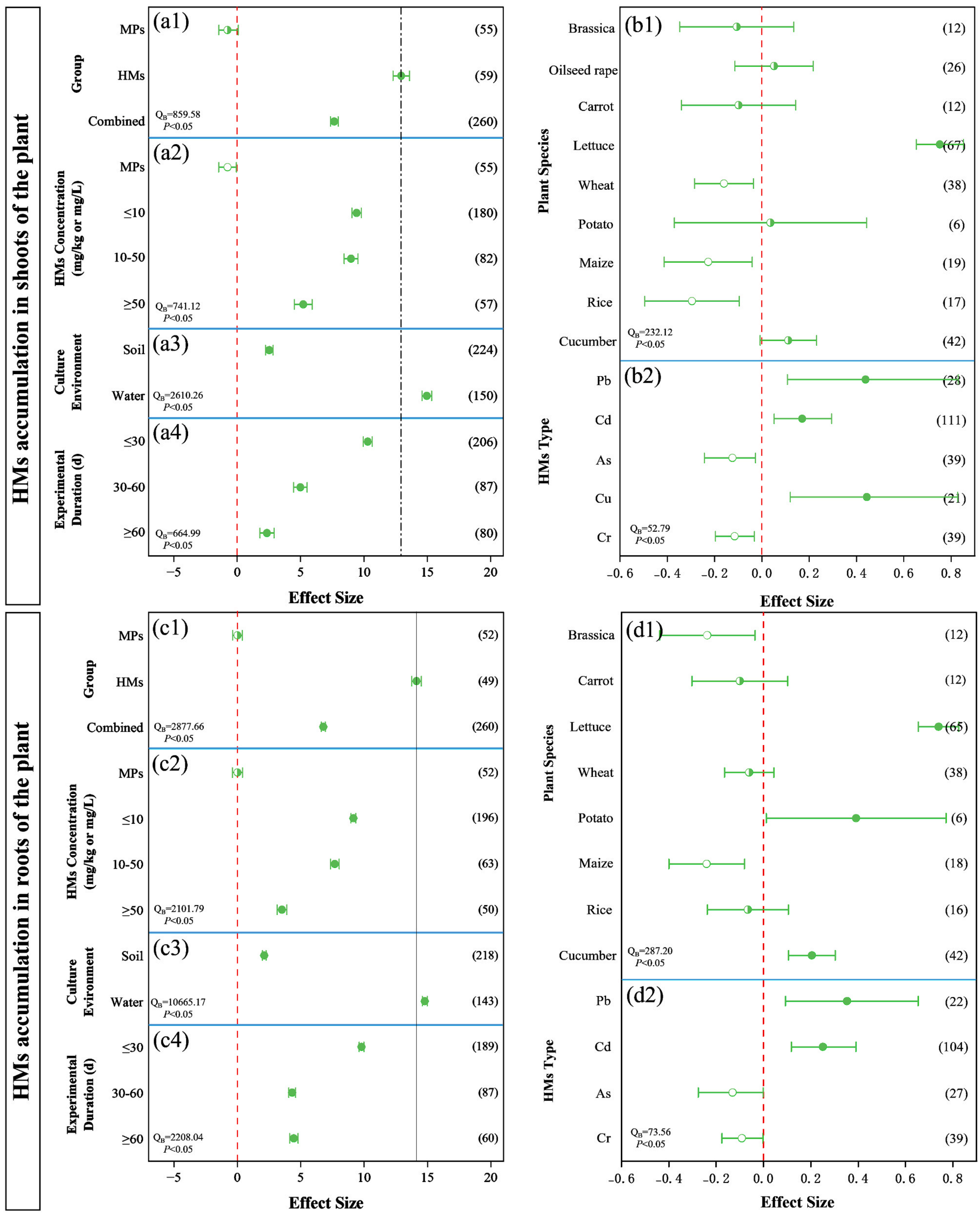

Fig. 4. Effect size of HMs characteristics and experimental methods on HMs accumulation in shoots and roots. The green points represent the effect size, and the error bars on either side indicate a 95 % confidence interval (CI) for the weighted mean difference combining the effect size. Red line, which effect size is zero, is used to help determine changes in the effect. Black line, the effect size for single HMs, is used to help compare its differences with single MPs and combined pollution. The overlap of the 95 % CI with the red line means a neutral effect, a positive effect entirely to its right and a negative effect entirely to its left. Solid points represent a positive effect, while semi-open and open points represent a neutral and negative effect, respectively. The numbers in parentheses are the amount of data included in the analysis. Q_B represents inter-group heterogeneity, and $P < 0.05$ indicates statistical significance. Con, conventional; Bio, biodegradable.

of HMs within a certain range. However, the high concentration of HMs can inhibit the growth and development of plants when they exceed the tolerance level of plants, which might be the reason for the relatively low HMs accumulation of plants under such conditions.

At the same time, different HMs can be absorbed by the roots in different ways. Subsequently, HMs are transported from roots to shoots via xylem or phloem, where they accumulation. Therefore, in order to further clarify the effects of MPs on the accumulation of different types of HMs in plants, combined pollution and single HMs pollution were compared. The results revealed that the accumulation of As and Cr in plants was inhibited in the presence of MPs (Figure 4b2 and d2), which was consistent with Li et al. results [22]. Apart from their adsorption to plant roots, MPs can also hinder HMs accumulation in plants through their unique ability to adsorb HMs. Metal ions that are adsorbed on the surface of MPs frequently have a low desorption rate and combine with MPs for a long time, which partially prevents plants from absorbing HMs [59]. However, the accumulation of Pb, Cd and Cu were enhanced by MPs (Figure 4b2 and d2). Several investigations have demonstrated that MPs can raise the risk of toxicity to plants by enhancing the bioavailability of HMs in soil [60,61]. Moreover, our previous meta-analysis also showed that MPs significantly enhance the bioavailability of Cu, Pb and Cd in soil, which may simultaneously increase their accumulation in plants [62]. In addition, plant root secretions and microbial communities may also promote their absorption. It was noted that the difference in electronegativity could also explain why the effects of MPs on As and Cr were different from Pb, Cd and Cu [22,63]. Arsenic and Cr, which have more chemical valence states, are often found in the natural environment in the form of anions, allowing MPs to increase their adsorption by changing the pH in soil [64], or reduce their accumulation in plants by adsorbing As and Cr via hydrogen bonds on the surface of MPs [65].

3.3.3. Experimental methods

The results revealed that soil culture was more effective than water culture in inhibiting HMs accumulation in shoots and roots (Figure 4a3 and c3). This might be due to the fact that complex environmental factors present in soils compared to water, including pH, organic matter concentration, and soil texture. Researches have shown that larger particles [66], lower pH levels [67], and less organic matter [68] might enhance HMs bioavailability in soil, making it easier for plants to absorb and accumulate. Furthermore, our previous research also found that there was a strong interaction between various factors in the soil that will intensify the influence of environmental factors on HMs bioavailability [62]. The water environment was relatively simple with less complex interactions. HMs pollutants in water were mostly added by humans, resulting in a higher HMs bioavailability compared to soil. However, MPs require a specific period to reach the roots of plants through water, which leads to the inhibition effect was not obvious in the short duration (Figure 4a4 and c4). Moreover, plants defense mechanisms are triggered when they exposure to MPs and HMs, causing the production of antioxidant kinase proteins, plant secretions, and phytochelatins to combat the harmful effects of MPs and HMs [3,27]. But it may take some time for the plant to adapt to this combined polluted environment, so in the short duration (≤30 d) the plant's resistance to HMs was not strong, resulting in relatively high absorption, which then gradually reduce over time. This might be why the inhibition in the root was no longer enhanced at the latter stage (Figure 4c4).

In addition, not all plants exhibited sensitivity to MPs during HMs accumulation. Among them, MPs did not affect HMs accumulation in brassica and carrot, but increased HMs accumulation in lettuce, potato and cucumber (Figure 4b1 and d1), and inhibited wheat, maize and rice. Some researchers have found that HMs accumulation in plants seemed to follow the rule of leafy>stalk/root/solanaceous>legume/melon, but the effect of MPs on plants did not seem to follow this pattern [69]. The inhibition effect of MPs on HMs accumulation in plants seemed to be related to the architectural root traits. Fibrous-root plants like maize and rice showed more significant inhibition of HM accumulation by MPs compared to thick-root plants such as brassica, carrot, lettuce, cucumber, and potato. Fibrous-root plants have higher root branching strength, a bigger number of roots per plant, and a faster root growth rate compared to thick-rooted plants, which may provide larger adsorption area for MPs, enhancing their resistance to HMs accumulation [70]. However, further experimental investigations are necessary to validate this hypothesis.

3.4. The key influencing factors and their interaction

According to the results of the relative importance of influencing factors (Fig. 5), the key influencing factors for shoots and roots were similar, with the top five were culture environment, HMs type, experimental duration, MPs concentration and MPs size. Based on the quantitative data of the key influencing factors, namely experimental duration, MPs concentration and MPs size, correlation analysis was conducted to further confirm the effect trend of each index suggested in the meta-analysis. The results showed that HMs accumulation in shoots and roots were negatively correlated with experimental duration, MPs concentration and MPs size ($P < 0.05$). In addition, although the importance of HMs concentration was relatively weak, it was statistically significant, and meta-analysis results also suggested that it could negatively affect HMs accumulation in shoots and roots, so the correlation between them was also analyzed. The correlation results showed that the HMs concentration was significantly negatively correlated with HMs accumulation in roots, while not in shoots (Fig. S4).

The results of meta-analysis on the comprehensive effects of combined pollution on plants have been suggested that the effect of HMs single pollution was more similar to combined pollution than that of MPs, indicating that HMs could have a significant influence on the effects of combined pollution on plant physiological indexes, while MPs were relatively weak. Among them, the combined pollution of MPs and HMs was more detrimental to the antioxidant stress ability and photosynthetic efficiency of plants themselves, but could reduce the effect of HMs on plant biomass to a certain extent (Fig. 6). This indicated that there may be an interaction between MPs and HMs. In order to test this hypothesis, GeoDetectors were used. The results showed that there were enhancement effects among all factors (Fig. 7a and b, and Table S3), among which the *Enhance, nonlinear-* interaction of each factor on HMs accumulation in shoots accounted for more (11.11 %), but relatively less in roots (5.56 %). At the same time, although MPs biodegradation was weak on its own, there was a clear interaction between it and other factors. The *Enhance, nonlinear-* interaction mainly existed between MPs biodegradation and the factors of HMs type and plant species on HMs accumulation in shoots and roots (Fig. 7c and d, Table S3). Biodegradable MPs aged more quickly than conventional MPs, providing them with more specific surface area to adsorb pollutants and microorganisms. Upon the formation of its surface biofilm, additional functional groups like -COOH and -NH- are provided to improve the adsorption of pollutants [71]. Meanwhile, biodegradable MPs were degraded through mechanical wear or microbial metabolism, resulting in the quicker production of nanoplastics compared to conventional MPs. In addition, biodegradable MPs were also considered as an organic carbon source that can affect environmental physicochemical factors and microbial community components in soil or water environments, thereby affecting HMs distribution in the environment [72]. These mechanisms, whether direct or indirect, might explain the stronger interaction between MPs biodegradation and other factors. However, this strong correlation also introduces uncertainties regarding the environmental risks it presents. Although there are limited studies on the phytotoxicity of biodegradable and conventional MPs, some studies have shown that biodegradable MPs are more conducive to the conversion of As in paddy soils into a bioavailable form [56].

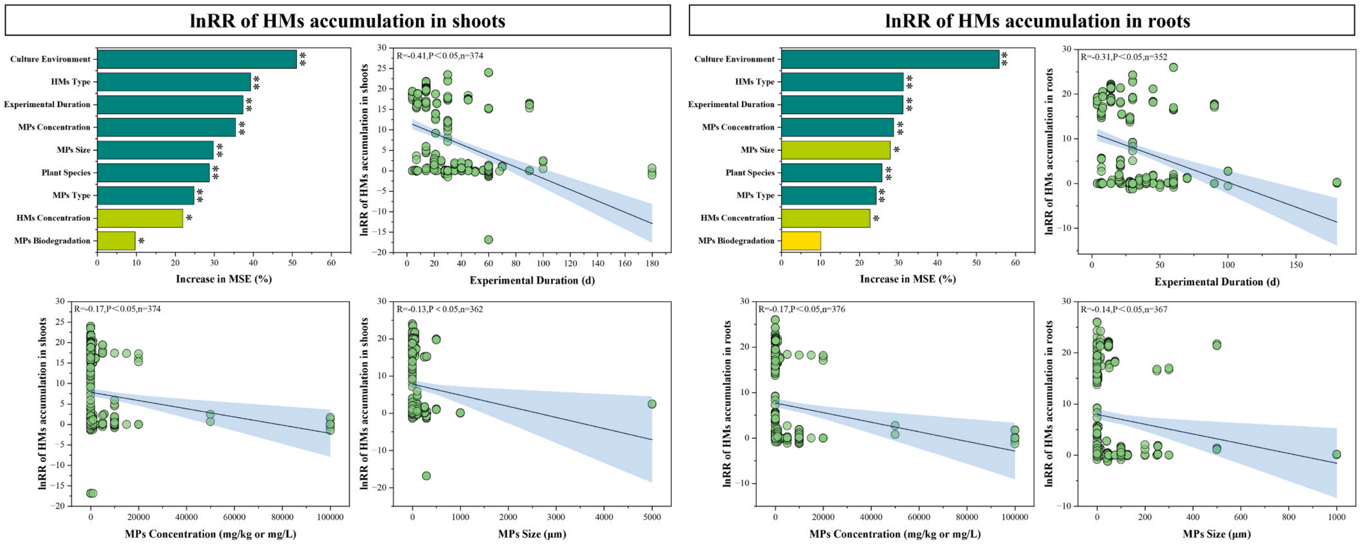

Fig. 5. The relative importance of influencing factors mediating the effects of combined pollution on HMs accumulation in shoots and roots, and their correlation analysis. R is the correlation coefficient, whose positive and negative indicates the direction of the effect, and the size indicates the strength of the effect. $P < 0.05$ indicates statistical significance. * $P < 0.05$, * * $P < 0.01$. MSE, mean squared error.

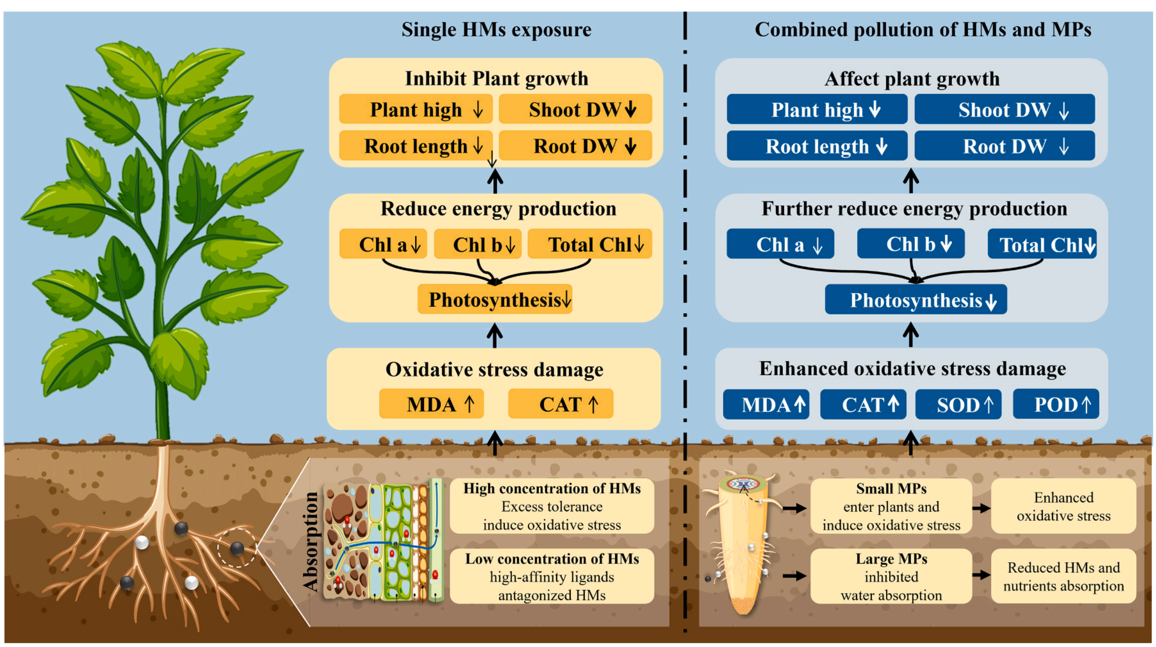

Fig. 6. The mechanism conceptual diagram illustrating how combined pollution of MPs and HMs affects the toxicity of plants. The direction of the arrow indicates an increase or decrease in the effects of combined pollution of MPs and HMs compared to single HMs, and the thickness indicates strong or weak. DW, dry weight; Chl, chlorophyll.

4. Environmental implications and limitations

By analyzing complex connection between the effects of combined pollution of MPs and HMs on HMs accumulation in plants, the results of this study suggested that, although MPs could reduce HMs accumulation in plants, this did not mean that their pollution should not be taken seriously. Because MPs may interact with HMs together to increase the toxicity of plants, affecting their quality and yield. For crops, this would reduce their economic efficiency and nutritional value, which is not conducive to food security and sustainable agricultural development. At the same time, this would also weaken the repair effectiveness of HMs hyperaccumulator, increasing the time and economic cost of phytoremediation. In addition, biodegradable MPs was considered as an alternative to conventional MPs, and although it reduced HMs accumulation in plants to a certain extent, its strong interaction with various factors indicated the importance of considering its environmental risks before promoting its use.

This research evaluated the combined effects of MPs and HMs on phytotoxicity, and revealed that there is an interaction between the two, which could jointly induce oxidative stress damage and threaten plant health. Unfortunately, phytotoxicity research on the combined pollution of MPs and HMs is lacking homogeneity, resulting in a fragmented data distribution. Insufficient data hindered subgroups analysis based on different MPs characteristics related to toxicity markers, making it difficult to conduct more in-depth exploration of the mechanism of combined phytotoxicity and screening of high environmental risk MPs.

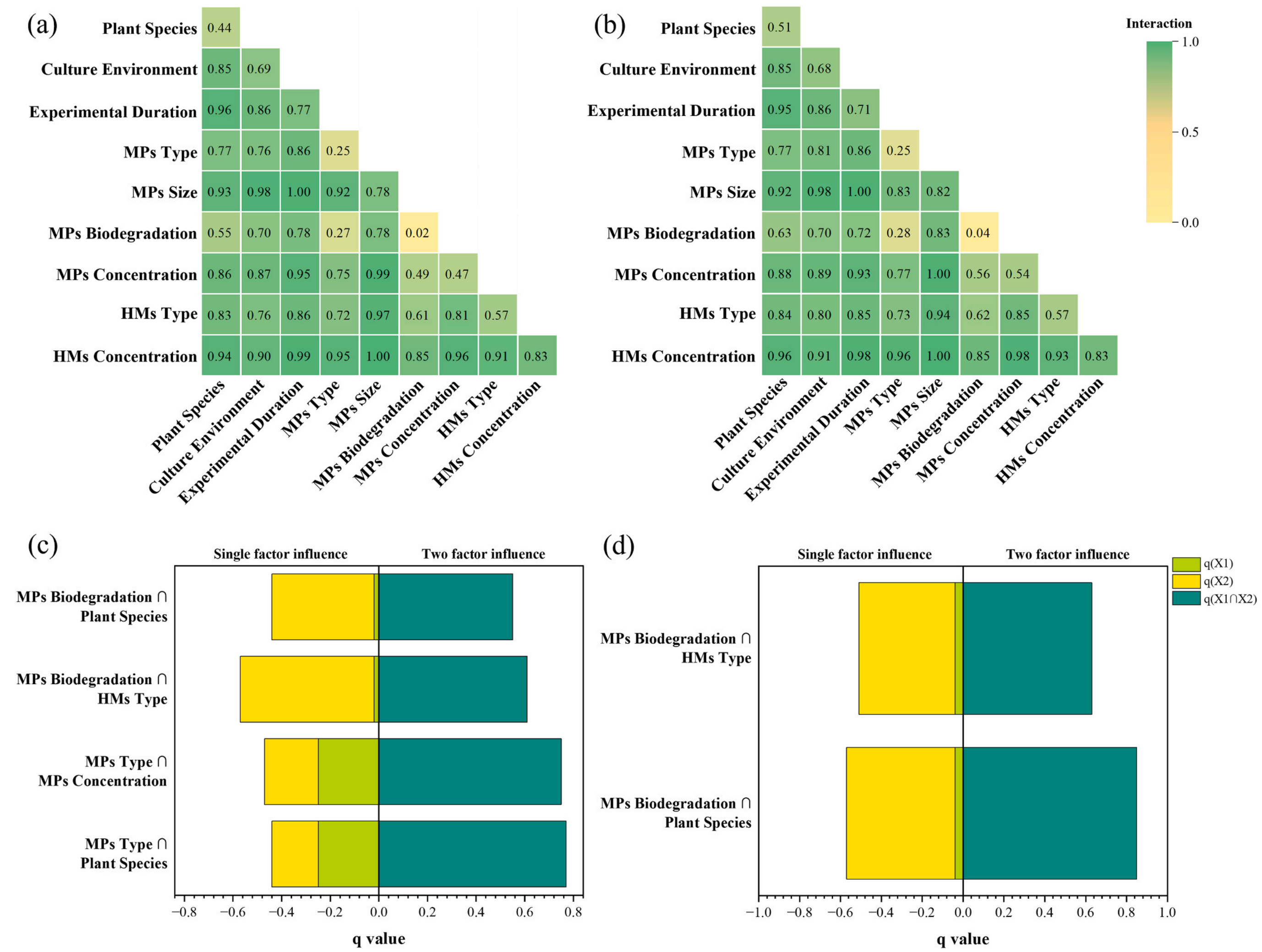

Fig. 7. The interaction between factors influencing HMs accumulation in shoots (a) and roots (b), and the value of all *Enhance, nonlinear*- interactions of HMs accumulation in shoots (c) and roots (d). ∩ represents the presence of an interaction between two factors.

In addition, microorganisms also contribute significantly to plant growth and metabolism, degradation and aging of MPs, and migration and transformation of HMs. However, few studies of the combined phytotoxicity of MPs and HMs have considered microbial community changes, making it impossible to analyze them.

5. Conclusion and future prospects

The ecological and environmental risks caused by the combined pollution of MPs and HMs have been one of the major concerns in the environmental field. However, due to the multiple characteristics of MPs and HMs and the complexity of combined pollution, it was still difficult to comprehensively identify the main influencing factors and explore their interaction. Therefore, this study compared the individual and combined effects of MPs and HMs on plants through data integration and statistical analysis. The results indicated that the combined toxic effects of MPs and HMs on plants are mainly induced by enhancement of oxidative stress damage, rather than increasing HMs accumulation in plants. Microplastics, especially those with larger sizes and high concentration, have been shown to inhibit rather than promote HMs accumulation in plants. Among them, conventional MPs have stronger inhibitory effect on HMs accumulation in plants than biodegradable MPs, while MPs biodegradation had stronger interactions with other factors. Culture environment, HMs type, experimental duration, MPs concentration and MPs size were the main factors affecting HMs accumulation in plants under the combined pollution of MPs and HMs. In general, the findings of this study provided some guidance for future environmental risk research on combined pollution of MPs and HMs, and also provided important insights into the toxicological effects of MPs com HMs on plants. However, some aspects seem to need further research in the future. More research about combined pollution of MPs and HMs should be conducted in order to support subgroup analysis in identifying high-risk MPs. Furthermore, the study of MPs translocation in plants will also benefit from increasing researches and development in quantitative technologies and MPs visualization. Meanwhile, studies on phytotoxicity of combined pollution were focused mostly on growth and enzyme activity indexes, which are relatively simple. High-throughput sequencing techniques such as transcriptomics, proteomics, and metabolomics can be considered for more in-depth exploration of toxic mechanisms.

Environmental implication

Because plants can transmit accumulated contaminants via the food chain, it is necessary to research the effects of MPs and HMs pollution on phytotoxicity. However, the results of previous studies in this field appear to be contradictory. This study employed meta-analysis to comprehensively investigate the effects of combined pollution of MPs and HMs on plants, identify the key influencing factors and assess whether there was interaction. This will help to evaluate the potential environmental risks caused by their combined pollution and has certain reference value for the development of MPs pollution management measures and remediation technologies.

CRediT authorship contribution statement

Qiuying An: Writing – original draft, Visualization, Software, Methodology, Investigation, Data curation. **Ce Wen:** Visualization, Investigation. **Changzhou Yan:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.135028.

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